

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1-18. Cancelled.

19. (New) A method for constructing a single chain diabody library, the method comprising:

(a) providing a first antibody library, each member of the library comprising a nucleotide sequence encoding a light chain variable domain and a nucleotide sequence encoding a heavy chain variable domain, both domains being directed against a first antigen, wherein the nucleotide sequence encoding the light chain variable domain is connected to the nucleotide sequence encoding the heavy chain variable domain by a first nucleotide linker of 30 to 150 base pairs comprising a cleavage site for a restriction enzyme;

(b) providing a second antibody library, each member of the library comprising (i) a nucleotide sequence encoding a light chain variable domain and (ii) a nucleotide sequence encoding a heavy chain variable domain, both of which domains are directed against a second antigen, wherein the nucleotide sequence of (i) is connected to the nucleotide sequence of (ii) by a second nucleotide linker of 30 to 150 base pairs, and wherein the end of the nucleotide sequence of (i) that is distal to the second linker and the end of the nucleotide sequence of (ii) that is distal to the second linker each comprises a cleavage site for the restriction enzyme;

(c) treating the first library with the restriction enzyme to cleave within the first linker;

(d) treating the second library with the restriction enzyme to produce a plurality of fragments, each fragment having a cleaved restriction site at its 5' end and its 3' end, wherein

each fragment comprises the nucleotide sequence of (b)(i) connected to the nucleotide sequence of (b)(ii) via the second linker; and

(e) ligating the cleaved product of (c) with the plurality of fragments of (d) to construct a third library of nucleic acids, each encoding a single polypeptide chain comprising both the light and heavy chain variable domains directed against the first antigen and the light and heavy chain variable domains directed against the second antigen, wherein the heavy and light chain variable domains directed against the second antigen are inserted between the light and heavy chain variable domains directed against the first antigen.

20. (New) The method of claim 19, wherein the antibody library is a phage antibody library.

21. (New) A method for constructing a single chain diabody library, the method comprising:

(a) providing a first antibody library, each member of the library comprising a nucleotide sequence encoding a light chain variable domain and a nucleotide sequence encoding a heavy chain variable domain, both domains being directed against a first antigen, wherein the nucleotide sequence encoding the light chain variable domain is connected to the nucleotide sequence encoding the heavy chain variable domain by a first nucleotide linker of 30 to 150 base pairs comprising a cleavage site for a first restriction enzyme and a cleavage site for a second restriction enzyme that is different from the first restriction enzyme;

(b) providing a second antibody library, each member of the library comprising (i) a nucleotide sequence encoding a light chain variable domain and (ii) a nucleotide sequence encoding a heavy chain variable domain, both of which domains are directed against a second antigen, wherein the nucleotide sequence of (i) is connected to the nucleotide sequence of (ii) by a second nucleotide linker of 30 to 150 base pairs, and wherein the end of the nucleotide sequence of (i) that is distal to the second linker comprises a cleavage site for the first restriction enzyme, and the end of the nucleotide sequence of (ii) that is distal to the second linker

comprises a cleavage site for the second restriction enzyme;

(c) treating the first library with the two restriction enzymes to cleave the two sites within the first linker;

(d) treating the second library with the two restriction enzymes to produce a plurality of fragments, each fragment having a cleaved restriction site at its 5' end and a cleaved restriction site at its 3' end, wherein each fragment comprises the nucleotide sequence of (b)(i) connected to the nucleotide sequence of (b)(ii) via the second linker; and

(e) ligating the cleaved product of (c) with the plurality of fragments of (d) to construct a third library of nucleic acids, each encoding a single polypeptide chain comprising both the light and heavy chain variable domains directed against the first antigen and the light and heavy chain variable domains directed against the second antigen, wherein the light and heavy chain variable domains directed against the second antigen are inserted between the light and heavy chain variable domains directed against the first antigen.

22. (New) The method of claim 21, wherein the antibody library is a phage antibody library.

23. (New) A method for constructing a single chain diabody library, the method comprising:

(a) providing a first antibody library, each member of the library comprising a nucleotide sequence encoding a first antibody variable domain and second antibody variable domain, wherein the nucleotide sequences encoding the first antibody variable domain is connected to the nucleotide sequence encoding the second antibody variable domain by a first nucleotide linker of 30 to 150 base pairs comprising a cleavage site for a restriction enzyme;

(b) providing a second antibody library, each member of the library comprising (i) a nucleotide sequence encoding a third antibody variable domain and (ii) a nucleotide sequence encoding a fourth antibody variable domain, wherein the nucleotide sequence of (i) is connected to the nucleotide sequence of (ii) by a second nucleotide linker of 30 to 150 base pairs, and

wherein the end of the nucleotide sequence of (i) that is distal to the second linker and the end of the nucleotide sequence of (ii) that is distal to the second linker each comprises a cleavage site for the restriction enzyme;

(c) treating the first library with the restriction enzyme to cleave within the first linker;

(d) treating the second library with the restriction enzyme to produce a plurality of fragments, each fragment having a cleaved restriction site at its 5' end and its 3' end, wherein each fragment comprises the nucleotide sequence of (b)(i) connected to the nucleotide sequence of (b)(ii) via the second linker; and;

(e) ligating the cleaved product of (c) with the plurality of fragments of (d) to construct a third library of nucleic acids, each encoding a single polypeptide chain comprising the first, second, third and fourth antibody variable domains, wherein the third and fourth antibody variable domains are inserted between the first and second antibody variable domains.

24. (New) The method of claim 23, wherein the antibody library is a phage antibody library.

25. (New) The method of claim 23, wherein the first and second antibody variable domains are directed against a first antigen and the third and fourth antibody variable domains are directed against a second antigen.

26. (New) The method of claim 23, wherein the first, second, third and fourth antibody variable domains are all directed against the same antigen.

27. (New) A method for constructing a single chain diabody library, the method comprising:

(a) providing a first antibody library, each member of the library comprising a nucleotide sequence encoding a first antibody variable domain and a nucleotide sequence

encoding a second antibody variable domain, both domains being directed against a first antigen, wherein the nucleotide sequence encoding the first antibody variable domain is connected to the nucleotide sequence encoding the second antibody variable domain by a first nucleotide linker of 30 to 150 base pairs comprising a cleavage site for a first restriction enzyme and a cleavage site for a second restriction enzyme that is different from the first restriction enzyme;

(b) providing a second antibody library, each member of the library comprising (i) a nucleotide sequence encoding a third antibody variable domain and (ii) a nucleotide sequence encoding a fourth antibody variable domain, both of which domains are directed against a second antigen, wherein the nucleotide sequence of (i) is connected to the nucleotide sequence of (ii) by a second nucleotide linker of 30 to 150 base pairs, and wherein the end of the nucleotide sequence of (i) that is distal to the second linker comprises a cleavage site for the first restriction enzyme, and the end of the nucleotide sequence of (ii) that is distal to the second linker comprises a cleavage site for the second restriction enzyme;

(c) treating the first library with the two restriction enzymes to cleave the two sites within the first linker;

(d) treating the second library with the two restriction enzymes to produce a plurality of fragments, each fragment having a cleaved restriction site at its 5' end and a cleaved restriction site at its 3' end, wherein each fragment comprises the nucleotide sequence of (b)(i) connected to the nucleotide sequence of (b)(ii) via the second linker; and

(e) ligating the cleaved product of (c) with the plurality of fragments of (d) to construct a third library of nucleic acids, each encoding a single polypeptide chain comprising the first, second, third and fourth antibody variable domains, wherein the third and fourth antibody variable domains are inserted between the first and second antibody variable domains.

28. (New) The method of claim 27, wherein the antibody library is a phage antibody library.

29. (New) The method of claim 27, wherein the first and second antibody variable

domains are directed against a first antigen and the third and fourth antibody variable domains are directed against a second antigen.

30. (New) The method of claim 27, wherein the first, second, third and fourth antibody variable domains are all directed against the same antigen.

31. (New) A method for producing a construct encoding a single chain diabody, the method comprising:

(a) providing a first nucleic acid comprising a nucleotide sequence encoding a light chain variable domain and a nucleotide sequence encoding a heavy chain variable domain, both domains being directed against a first antigen, wherein the nucleotide sequence encoding the light chain variable domain is connected to the nucleotide sequence encoding the heavy chain variable domain by a first nucleotide linker of 30 to 150 base pairs comprising a cleavage site for a restriction enzyme;

(b) providing a second nucleic acid comprising (i) a nucleotide sequence encoding a light chain variable domain and (ii) a nucleotide sequence encoding a heavy chain variable domain, both of which domains are directed against a second antigen, wherein the nucleotide sequence of (i) is connected to the nucleotide sequence of (ii) by a second nucleotide linker of 30 to 150 base pairs, and wherein the end of the nucleotide sequence of (i) that is distal to the second linker and the end of the nucleotide sequence of (ii) that is distal to the second linker each comprises a cleavage site for the restriction enzyme;

(c) treating the first nucleic acid with the restriction enzyme to cleave within the first linker;

(d) treating the second nucleic acid with the restriction enzyme to produce a fragment having a cleaved restriction site at its 5' end and its 3' end, wherein the fragment comprises the nucleotide sequence of (b)(i) connected to the nucleotide sequence of (b)(ii) via the second linker; and

(e) ligating the cleaved product of (c) with the fragment of (d) to construct a third

nucleic acid encoding a single polypeptide chain comprising the light and heavy chain variable domains directed against the first antigen and the light and heavy chain variable domains directed against the second antigen, wherein the light and heavy chain variable domains directed against the second antigen are inserted between the light and heavy chain variable domains directed against the first antigen.

32. (New) The method of claim 31, wherein the third nucleic acid either (a) is an expression vector or (b) is inserted into an expression vector subsequent to the ligating step.

33. (New) A method for producing a construct encoding a single chain diabody, the method comprising:

(a) providing a first nucleic acid comprising a nucleotide sequence encoding a light chain variable domain and a nucleotide sequence encoding a heavy chain variable domain, both domains being directed against a first antigen, wherein the nucleotide sequence encoding the light chain variable domain is connected to the nucleotide sequence encoding the heavy chain variable domain by a first nucleotide linker of 30 to 150 base pairs comprising a cleavage site for a first restriction enzyme and a cleavage site for a second restriction enzyme that is different from the first restriction enzyme;

(b) providing a second nucleic acid comprising (i) a nucleotide sequence encoding a light chain variable domain and (ii) a nucleotide sequence encoding a heavy chain variable domain, both of which domains are directed against a second antigen, wherein the nucleotide sequence of (i) is connected to the nucleotide sequence of (ii) by a second nucleotide linker of 30 to 150 base pairs, and wherein the end of the nucleotide sequence of (i) that is distal to the second linker comprises a cleavage site for the first restriction enzyme, and the end of the nucleotide sequence of (ii) that is distal to the second linker comprises a cleavage site for the second restriction enzyme;

(c) treating the first nucleic acid with the two restriction enzymes to cleave the two sites within the first linker;

(d) treating the second nucleic acid with the two restriction enzymes to produce a fragment having a cleaved restriction site at its 5' end and a cleaved restriction site at its 3' end, wherein the fragment comprises the nucleotide sequence of (b)(i) connected to the nucleotide sequence of (b)(ii) via the second linker; and

(e) ligating the cleaved product of (c) with the fragment of (d) to construct a third nucleic acid encoding a single polypeptide chain comprising both the light and heavy chain variable domains directed against the first antigen and the light and heavy chain variable domains directed against the second antigen, wherein the light and heavy chain variable domains directed against the second antigen are inserted between the light and heavy chain variable domains directed against the first antigen.

34. (New) The method of claim 33, wherein the third nucleic acid either (a) is an expression vector or (b) is inserted into an expression vector subsequent to the ligating step.

35. (New) A method for producing a construct encoding a single chain diabody, the method comprising:

(a) providing a first nucleic acid comprising a nucleotide sequence encoding a first antibody variable domain and a nucleotide sequence encoding a second antibody variable domain, both domains being directed against a first antigen, wherein the nucleotide sequence encoding the first antibody variable domain is connected to the nucleotide sequence encoding the second antibody variable domain by a first nucleotide linker of 30 to 150 base pairs comprising a cleavage site for a restriction enzyme;

(b) providing a second nucleic acid comprising (i) a nucleotide sequence encoding a third antibody variable domain and (ii) a nucleotide sequence encoding a fourth antibody variable domain, both of which domains are directed against a second antigen, wherein the nucleotide sequence of (i) is connected to the nucleotide sequence of (ii) by a second nucleotide linker of 30 to 150 base pairs, and wherein the end of the nucleotide sequence of (i) that is distal to the second linker and the end of the nucleotide sequence of (ii) that is distal to the second

linker each comprises a cleavage site for the restriction enzyme;

(c) treating the first nucleic acid with the restriction enzyme to cleave within the first linker;

(d) treating the second nucleic acid with the restriction enzyme to produce a fragment having a cleaved restriction site at its 5' end and its 3' end, wherein the fragment comprises the nucleotide sequence of (b)(i) connected to the nucleotide sequence of (b)(ii) via the second linker; and

(e) ligating the cleaved product of (c) with the fragment of (d) to construct a third nucleic acid encoding a single polypeptide chain comprising the first, second, third and fourth antibody variable domains, wherein the third and fourth antibody variable domains are inserted between the first and second antibody variable domains.

36. (New) The method of claim 35, wherein the third nucleic acid either (a) is an expression vector or (b) is inserted into an expression vector subsequent to the ligating step.

37. (New) The method of claim 35, wherein the first and second antibody variable domains are directed against a first antigen and the third and fourth antibody variable domains are directed against a second antigen.

38. (New) The method of claim 35, wherein the first, second, third and fourth antibody variable domains are all directed against the same antigen.

39. (New) A method for producing a construct encoding a single chain diabody, the method comprising:

(a) providing a first nucleic acid comprising a nucleotide sequence encoding a first antibody variable domain and a nucleotide sequence encoding a second antibody variable domain, both domains being directed against a first antigen, wherein the nucleotide sequence encoding the first antibody variable domain is connected to the nucleotide sequence encoding the

second antibody variable domain by a first nucleotide linker of 30 to 150 base pairs comprising a cleavage site for a first restriction enzyme and a cleavage site for a second restriction enzyme that is different from the first restriction enzyme;

(b) providing a second nucleic acid comprising (i) a nucleotide sequence encoding a third antibody variable domain and (ii) a nucleotide sequence encoding a fourth antibody variable domain, both of which domains are directed against a second antigen, wherein the nucleotide sequence of (i) is connected to the nucleotide sequence of (ii) by a second nucleotide linker of 30 to 150 base pairs, and wherein the end of the nucleotide sequence of (i) that is distal to the second linker comprises a cleavage site for the first restriction enzyme, and the end of the nucleotide sequence of (ii) that is distal to the second linker comprises a cleavage site for the second restriction enzyme;

(c) treating the first nucleic acid with the two restriction enzymes to cleave the two sites within the first linker;

(d) treating the second nucleic acid with the two restriction enzymes to produce a fragment having a cleaved restriction site at its 5' end and a cleaved restriction site at its 3' end, wherein the fragment comprises the nucleotide sequence of (b)(i) connected to the nucleotide sequence of (b)(ii) via the second linker; and

(e) ligating the cleaved product of (c) with the fragment of (d) to construct a third nucleic acid encoding a single polypeptide chain comprising the first, second, third and fourth antibody variable domains, wherein the third and fourth antibody variable domains are inserted between the first and second antibody variable domains.

40. (New) The method of claim 39, wherein the third nucleic acid either (a) is an expression vector or (b) is inserted into an expression vector subsequent to the ligating step.

41. (New) The method of claim 39, wherein the first and second antibody variable domains are directed against a first antigen and the third and fourth antibody variable domains are directed against a second antigen.

42. (New) The method of claim 39, wherein the first, second, third and fourth antibody variable domains are all directed against the same antigen.

43. (New) A method for constructing an antibody library, the method comprising:

(a) providing an antibody library, each member of the library comprising a first nucleotide sequence encoding a light chain variable domain and a second nucleotide sequence encoding a heavy chain variable domain, both domains being directed against the same antigen, wherein the first nucleotide sequence is connected to the second nucleotide sequence by a linker of 30 to 150 base pairs comprising two or more cleavage sites for a restriction enzyme;

(b) treating the library with the restriction enzyme to cleave the two or more sites within the linker; and

(c) self-ligating the cleaved product of (b) to generate a second antibody library, each member of the second library comprising the first and the second nucleotide sequences joined by a linker that is shorter than the linker in the library of (a).

44. (New) The method of claim 43, wherein the antibody library is a phage antibody library.